# Evidence of relictual introgression or incomplete lineage sorting in nrDNA of *Juniperus excelsa* and *J. polycarpos* in Asia Minor

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## ABSTRACT

DNA analysis of *Juniperus excelsa* from throughout its range revealed that *J. polycarpos*, instead of *J. excelsa* occupies central and eastern Turkey. Based on nrDNA (ITS) data, it appears that relictual hybridization has occurred in southeastern Turkey between *J. polycarpos* and *J. turcomanica*. Surprisingly, evidence of incomplete lineage sorting or relictual hybridization between *J. polycarpos* and *J. seravschanica* was found in central Turkey and northwest Iran. Published on-line **www.phytologia.org** *Phytologia 98(2): 146-155 (April 4, 2016*).* ISSN 030319430 *digitally corrected-Adam Boratynski, and symbols added to Fig. 3, May, 10, 2016.

**KEY WORDS:** *Juniperus excelsa, J. polycarpos* var. *polycarpos, J. polycarpos* var. *turcomanica, J. seravschanica,* DNA sequencing, nrDNA, petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF.

Recently, Adams et al. (2016) examined *J. excelsa* and putative *J. polycarpos* from the eastern Mediterreanea, eastward into Azerbaijan. A Bayesian consensus tree shows *Juniperus seravschanica, J. polycarpos, J. p.* var. *turcomanica, J. procera* and *J. excelsa* in well-supported clades. *J. excelsa* samples, newly collected from Bulgaria, Cyprus, and sw Turkey, are in a clade with other *J. excelsa* (Fig. 1). There is some minor variation among the *J. excelsa* samples, mostly notably in the Afqa, Lebanon population as previously reported (Douaihy et al., 2011, 2013).

All of the *J. polycarpos* samples from Azerbaijan are closely related with *J. polycarpos*, Armenia along with the El Njass, Lebanon (*Adams 14161*) sample (Fig. 1). Three other El Njass samples (*Adams 14158, 14158, 14160*) appear to be intermediate between *J. polycarpos and J. p.* var. *turcomanica* (Fig. 1).

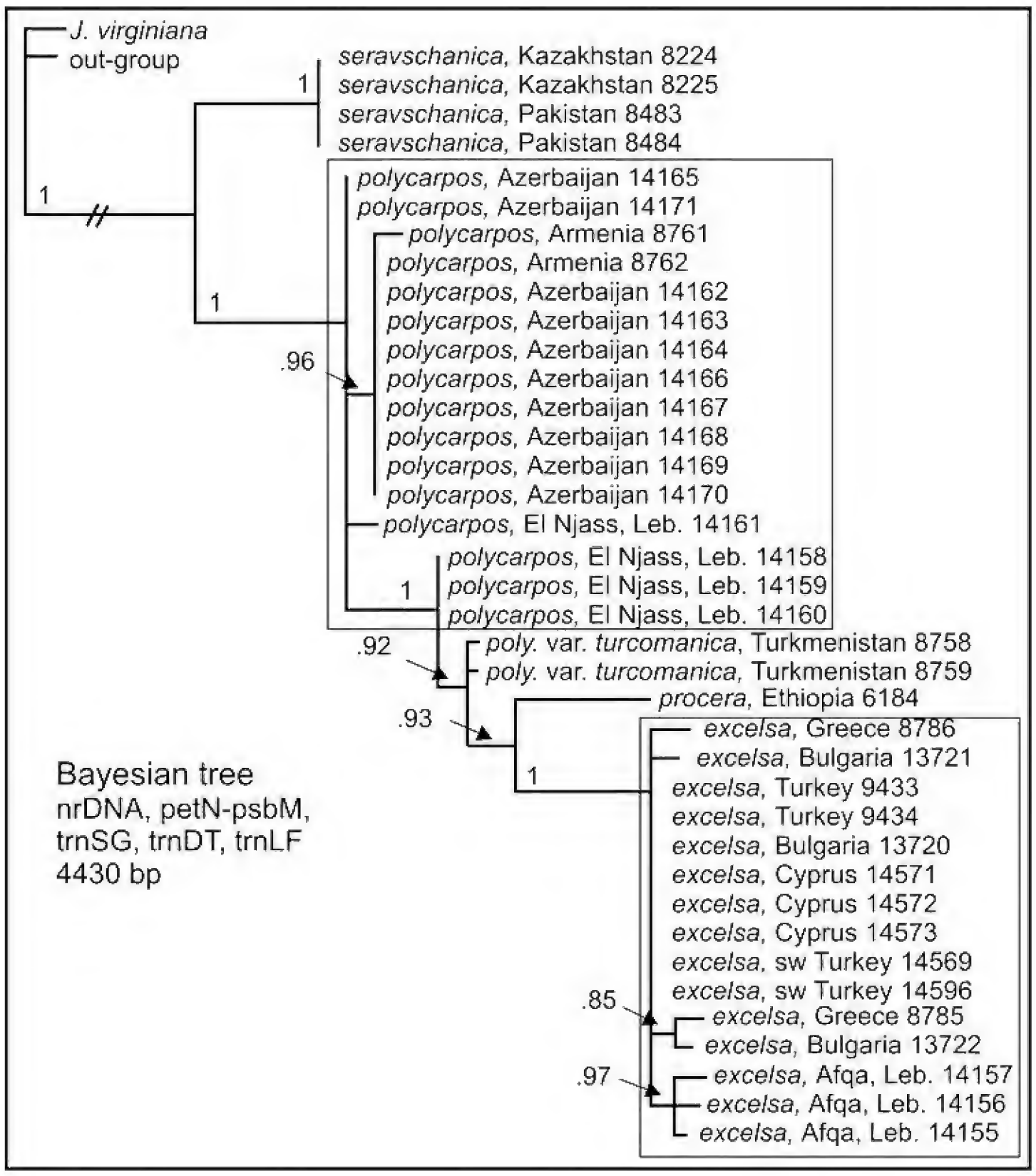


Figure 1. Bayesian analysis based on nrDNA, petN-psbM, trnSG, trnDT and trnLF. Numbers at the branch points are posterior probabilities.

Overlaying a minimum spanning network onto a distribution map gives one a perspective of the geographic trends (Fig. 2). The newly sampled *J. excelsa* populations from Bulgaria, Cyprus and sw

Turkey were identical or nearly identical to *J. excelsa* of Eskisehir, Turkey (Fig. 2). Both the Cyprus and southwestern Turkey populations of *J. excelsa* showed no differences (Fig. 2). The Bulgaria *J. excelsa* differed by none or one difference from Eskisehir, Turkey (Fig. 2).

As previously reported (Adams et al., 2014), the Afqa, Lebanon *J. excelsa* population differs by 2 MEs from Eskisehir, Turkey, which in turn, differs by only 1 ME from the Lemos, Greece population (Fig. 2). The other Lebanon populations that group with Afqa are probably *J. excelsa*.

However, the Wadi El Njass, Lebanon (2287 m) population, although near Afqa, grouped with *J. polycarpos* and differs by 1 to 3 MEs from *J. p.* var. *turcomanica*, Turkmenistan and by 1 to 2 MEs from *J. polycarpos,* Armenia (Fig. 2). The *J. excelsa*, Afqa population is only about 100 - 150 km from other *J. excelsa* populations (Fig. 2), but the Wadi El Njass, *J. polycarpos* population is 700 to 1000 km from the nearest *J. polycarpos* population, still, it differs by only 1 to 3 MEs.


Figure 2. Minimum spanning network mapped onto the distributions of *J. excelsa and J. polycarpos*. Numbers next to lines are the number of MEs (Mutational Events = base substitutions plus indels).

Adams et al. (2016) concluded that *J. excelsa*, as sampled in their study, was a fairly uniform species, except for the unusual situation in Lebanon, where *J. excelsa* and *J. polycarpos* (and likely *J. p.* var. *turcomanica*) grow near each other and may be hybridizing. However, the genetic composition of the eastern-most populations of *J. excelsa* in Turkey was unknown and deserved additional study.

Farjon (2005, 2010) treated *J. polycarpos, J. p.* var. *seravschanica* and *J. p.* var. *turcomanica* as *J. excelsa* subsp. *polycarpos*. However, Adams et al. (2008), Adams and Schwarzbach (2012) and Adams (2013, 2014), utilizing DNA sequence data, recognized *J. excelsa,* in addition to *J. polycarpos, J. p.* var.

*turcomanica* and *J. seravschanica.* Adams and Hojjati (2012) and Adams, Hojjati and Schwarzbach (2014), using sequences from 4 gene regions, did not find *J. excelsa* in Iran, but did confirm *J. polycarpos*, *J. p.* var. *turcomanica* and *J. seravschanica* in Iran. Putative *J. excelsa* from Qushchi, in extreme northwest Iran, had none or only one SNP difference compared with *J. polycarpos* var. *polycarpos* from Armenia and was concluded to be *J. polycarpos* (Adams and Hojjati, 2012). Adams et al. (2014) found that putative *J. excelsa* in Azerbaijan was, in fact, *J. polycarpos* or in one case, a putative hybrid (*polycarpos* x *turcomanica*).

The distribution of *J. excelsa* in eastern Turkey has proved difficult to determine by modern methods of DNA sequencing, due to the lack of samples from these regions. Recently, materials were obtained of *J. excelsa* from central and eastern Turkey. This afforded the opportunity to further examine geographic variation of *J. excelsa* and *J. polycarpos*.

## MATERIALS AND METHODS

**Plant material - *J. excelsa:***
**Bulgaria**, Central Rhodopes, above the town of Kritchim, Reserve "Izgorialoto Gune", 42° 01' 22.0" N; 24° 28' 03.1" E, 356 m, *Alex Tashev, 2012-1-JE -5-JE*, 1 Sep 2012, Lab Acc. *Adams 13720-13724*,; **Crimea:** Karadigski Zapovidnyk, between Kurortne and Koktebel, 44.914° N, 35.215° E. 220m, *A. Boratynski, Y. Didukh, K. Romashenko, A. Romo, A. Susanna, 2001, KOR 49898*, Karadag near Kolhoznoe, 44° 28' 06" N, 33° 49' 54" E, ca 530m; *A. Boratynski, G. Iszkulo, A. Lewandowski, 2006, KOR 45630*; **Cyprus**: 34° 57' 45.82" N, 33° 59' 55.33" E, 1461m, *Salih Gucel ns*, 3 July 2015, Lab Acc. *Adams 14570-14574*; **Greece**: Lemos, ca 40° 49' N, 21° 03' E, 1100m, *Adams 5983-5985, 5987*; **Lebanon**: Afqa, 34° 04' 58.12"N, 35° 53' 08.52" E, 1306 m, *Bouchra Douaihy 1-3*, 4 Nov 2013, Lab Acc. *Adams 14155-14157*; **Turkey**: Antalya-Manavgat, Köprülü Canyon National Park, 37° 20' N; 31° 16' E, 550 m, *Tuğrul Mataraci 2015-18*, 24 May 2015, Lab Acc *Adams 14569*; Isparta-Eğirdir, junction of Kasımlar-Sütçüler road, 37° 28' N; 30° 59' E, 1180 m, *Tugrul Mataraci, 2015-7*, 24 May 2015, Lab Acc. *Adams 14596*; ~40 km north of Eskişehir, with Oaks, 39° 58.307'N; 30° 41.045' E, Turkey, 820m, *Adams 9433-9435;* Sirnak, se Turkey on Turkey/Iraq border, 37° 34' 08" N, 43° 09' 45" E, 1754m, *Metin Armagan ns*, Lab. Acc. *Adams 14709-14712*, Sirnak Turkey, on Turkey/Iraq border se Anatolia, Prov., near Beytussebap, GE ca 37° 37' 18"N, 42 52' 28" E, 1420m, *Metin Armagan ns*, Lab. Acc. *Adams14715*; 3 km from Unlupinar village towards Gumushane, 40° 14' 25.14" N; 39° 27' 19.17" E. ne Turkey, 1763m, *Ali Kandemir 10846*, Lab Acc. *Adams 14713*; around Lake Ardicli, near Ergan Mountains, near Erzincan. 39° 37' 47.45" N; 39° 29' 54.3" E. ne Turkey. 1797m, *Ali Kandemir 10850*, Lab Acc. *Adams 14714*; Material from specimens at the herbarium, Yüzüncü Yıl University, Van, Turkey: *Adams 14750-* 1986, 38° 28' 41.7" N, 43° 31' 20.6" E, 2800m; *Adams 14751-* 1989, 38° 08' 37.5" N, 42° 17' 15.2" E, 1550m; *Adams 14752*, 1995, 38° 36' 20.2" N, 38° 47' 35.0" E, 1700m; *Adams 14753-* 1996, 38° 40' 6.6" N, 38° 44' 58.6" E, 1600m; *Adams 14754-* 2001, 38° 20' 30.7" N, 43° 37' 44.9" E, 2000m; *Adams 14755-* 2006, 39°13'8.08"N 43° 23' 4.11"E, 1965m; *Adams 14756-* 2014, 38°04'33.6"N 43° 25' 32.0"E, 2010m; *Adams 14757-* 2014, 39° 07' 47" N, 38° 47' 0.5" E, 1275m; *Adams 14758-* 2014, 39° 07' 50.6" N, 39° 07' 27.2" E, 1720m; *Adams 14759-* 2014, 39° 19' 42.4" N, 39° 25' 41.3" E, 1860m; *Adams 14760-* 2014, 39° 21' 17.6" N, 39° 28' 28.8" E, 1890m;
***J. polycarpos*:**
**Armenia**: Lake Sevan, 1900m, *Adams 8761-8763*; **Azerbaijan**, 40° 44' 41.05" N; 47° 35' 19.14" E, 177-231m, *Vahid Farzaliyev 1-10*, Dec 2013, Lab Acc. *Adams 14162-14171*; **Lebanon:**, Wadi El Njass, 34° 20' 47.79"N, 36° 05' 45.54"E, 2287m, *Bouchra Douaihy 4-7*, 14 Nov 2013, Lab Acc. *Adams 14158-14161*;
***J. polycarpos* var. *turcomanica*: Turkmenistan**: Kopet Mtns., 38° 25.12'N, 56° 58.80'E, 1535 m, 22 May 1999, *Adams 8758-8790*;
***J. procera*: Ethiopia**: on the road to Guder, ca. 40 km w of Addis Abba, ca. 9° 02'N, 38° 24' W, 2400 m, *Adams 6184-6188*;

***J. seravschanica*: Pakistan**: near Quetta, Baluchistan, *A. Hafeez Buzdar ns*, 6 Apr 1998, Lab Acc. *Adams 8483-8485*; **Kazakhstan**: west end of Talasskiy Ala-Tau Range, ca. 2 km S. of Dzhabagly, $42^0$ 24.53'N, $70^0$ 28.50'E, 1770m, 12 Sept 1997, *Adams 8224-8226*.
Voucher specimens deposited in the Herbarium, Baylor University (BAYLU).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions. Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN, trnD-T, trnL-F, trnS-G) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. The primers for trnD-trnT, trnL-trnF and trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010).

The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com). Sequence datasets were analyzed using Geneious v. R8 (Biomatters. Available from http://www.geneious.com/), the MAFFT alignment program. Further analyses utilized the Bayesian analysis software Mr. Bayes v.3.1 (Ronquist and Huelsenbeck 2003). For phylogenetic analyses, appropriate nucleotide substitution models were selected using Modeltest v3.7 (Posada and Crandall 1998) and Akaike's information criterion. Minimum spanning networks were constructed from mutational events (ME) data using PCODNA software (Adams et al., 2009; Adams, 1975; Veldman, 1967).

## RESULTS AND DISCUSSION

The classification of samples on the basis of ITS and petN (cp data) is given in Table 1. First it should be noted that nrDNA does not distinguish *J. excelsa* (exc) from *J. p.* var. *turcomanica* (tur) (compare 1st table entry, Greece A G C C C T A exc vs. Turkmenistan (last entry) A G C C C T A tur.). Secondly, exc (or tur) is very distinct from pol in its nrDNA (exc: A G C C C T A vs. pol: C G T T T T C T). Thirdly, exc.(or tur) is very distinct from ser (exc: A G C C C T A vs. ser: C G C T T T C T). And, finally, nrDNA for pol has only one nucleotide different from ser (pol: C G **T** T T T C T vs ser: C G **C** T T T C T). Several plants had nrDNA from one taxon, but cp DNA from another taxon: El Njass (3 E,P); Metin e Turkey, *14757*, (S,P); Azerbaijan *14171* (S,P); Elburz Mtn., Iran *12504* (S,P); Lushan, Iran *12798* (S,P); Hastjin, Iran *12795* (S,P); and Qushchi, Iran, *12798* (S,P). Other plants appeared to be hybrids by nrDNA: El Njass, *14161*, PxE; Metin e Turkey *14753*, PxS; Metin e Turkey *14754*, PxS; Metin e Turkey *14758*, PxS; ne Turkey 14714, PxS; se Turkey/ Iraq border *14715*, PxT(or E); se Turkey/ Iraq border *14709*, PxT(or E); se Turkey/ Iraq border *14710*, PxT(or E); and Azerbaijan, *14165*, PxT(or E).

To visualize this variation, plants were mapped with their nrDNA and cp (petN) DNA coded (Fig. 3). It is sometimes difficult to determine whether a variation is due to incomplete lineage sorting or hybridization (see discussion in Naciri and Linder, 2015). In the present study, the odd occurrence of *J. seravschanica* nrDNA in central-eastern Turkey plants seems more likely incomplete lineage sorting than hybridization, because no pure *J. seravschanica* grows sympatric with *J. polycarpos* in the area. Long distance cross-pollination is possible but unlikely as the nearest known *J. seravschanica* is quite distant (Fig. 4). In northwestern Iran one P,P and three S,P plants were found. This may be due to either hybridization or incomplete lineage sorting. Additional samples are needed to better understand that region.

Table 1. Classification of samples, on ITS and cp (petN) sequence data. exe = *excelsa*, pol = *polycarpos*, tur = *turcomanica*, ser = *seravschanica*. PxE = hybrid pol x exc; PxS = hybrid pol x ser; PxT(E) = pol x tur (or exc, as ITS for exe = tur).

| source | acc # | 230 | 232 | 238 | 354 | 427 | 732 | 8952 | ITS | cp |
|---|---|---|---|---|---|---|---|---|---|---|
| n Greece, 1010m | 8785 | A | G | C | C | C | T | A | exc | exc |
| n Greece, 1010m | 8786 | A | G | C | C | C | T | A | exc | exc |
| n Greece, 903m | 14742 | A | G | C | C | C | T | A | exc | exc |
| Bulgaria, 365m | 13720 | A | G | C | C | C | T | A | exc | exc |
| Bulgaria, 365m | 13721 | A | G | C | C | C | T | A | exc | exc |
| Bulgaria, 365m | 13722 | A | G | C | C | C | T | A | exc | exc |
| Cyprus, 1461m | 14570 | A | G | C | C | C | T | A | exc | exc |
| Cyprus, 1461m | 14571 | A | G | C | C | C | T | A | exc | exc |
| Cyprus, 1461m | 14572 | A | G | C | C | C | T | A | exc | exc |
| Crimea, 220m | 14906 | A | G | C | C | C | T | A | exc | exc |
| Crimea, 530m | 14907 | A | G | C | C | C | T | A | exc | exc |
| sw Turkey, 550m | 14569 | A | G | C | C | C | T | A | exc | exc |
| sw Turkey, 1461m | 14596 | A | G | C | C | C | T | A | exc | exc |
| nw Turkey, Eskisehir, 820m | 9433 | A | G | C | C | C | T | A | exc | exc |
| nw Turkey, Eskisehir, 820m | 9434 | A | G | C | C | C | T | A | exc | exc |
| Afqa Leb M1, 1306m | 14155 | A | G | C | C | C | T | A | exc | exc |
| Afqa Leb M2, 1306m | 14156 | A | G | C | C | C | T | A | exc | exc |
| Afqa Leb M3, 1306m | 14157 | A | G | C | C | C | T | A | exc | exc |
| El Njass Leb M4, 2287m | 14158 | A | G | C | C | C | T | A | exc | poly |
| El Njass Leb M5, 2287m | 14159 | A | G | C | C | C | T | A | exc | poly |
| El Njass Leb M5, 2287m | 14160 | A | G | C | C | C | T | A | exc | poly |
| El Njass Leb M7, 2287m | 14161 | **Y-C/T** | G | **Y-C/T** | C | **Y-C/T** | **Y-C/T** | **W-A/T** | **PxE** | poly |
| Metin e Turkey, 2800m | 14750 | na | na | na | na | T | C | T | poly | poly |
| Metin e Turkey, 1550m | 14751 | C | G | T | T | T | C | T | poly | poly |
| Metin e Turkey, 1700m | 14752 | C | G | T | T | T | C | T | poly | poly |
| **Metin e Turkey, 1600m** | **14753** | **C** | **G** | **T** | **Y-C/T** | **T** | **C** | **T** | **PxS** | **poly** |
| **Metin e Turkey, 2000m** | **14754** | **C** | **G** | **T** | **Y-C/T** | **T** | **C** | **T** | **PxS** | **poly** |
| Metin e Turkey, 1965m | 14755 | C | A | T | T | T | C | T | poly | poly |
| Metin e Turkey, 2010m | 14756 | C | G | T | T | T | C | T | poly | poly |
| Metin e Turkey, 1275m | 14757 | C | G | T | C | T | C | T | **serav** | poly |
| **Metin e Turkey, 1720m** | **14758** | **C** | **G** | **T** | **Y-C/T** | **T** | **C** | **T** | **PxS** | **poly** |
| Metin e Turkey, 1860m | 14759 | C | G | T | T | T | C | T | poly | poly |
| Metin e Turkey, 1890m | 14760 | C | G | T | T | T | C | T | poly | poly |
| ne Turkey , 1,753m | 14713 | C | G | T | T | T | C | T | poly | poly |
| ne Turkey, 1,783m | 14714 | C | **R-A/G** | T | **Y-C/T** | T | C | T | **PxS**[a] | poly |
| se Turkey/ n Iraq, 1,420m | 14715 | **Y-C/T** | G | **Y-C/T** | C | **Y-C/T** | **Y-C/T** | **W-A/T** | **PxT(E)** | poly |
| se Turkey/ n Iraq, 1,743m | 14709 | **Y-C/T** | G | **Y-C/T** | C | **Y-C/T** | **Y-C/T** | **W-A/T** | **PxT(E)** | poly |
| se Turkey/ n Iraq, 1,743m | 14710 | **Y-C/T** | G | **Y-C/T** | C | **Y-C/T** | **Y-C/T** | **W-A/T** | **PxT(E)** | poly |
| se Turkey/ n Iraq, 1,743m | 14711 | C | G | T | T | T | C | T | poly | poly |
| se Turkey/ n Iraq, 1,743m | 14712 | C | G | T | T | T | C | T | poly | poly |
| Azerbaijan, 200m | 14162 | C | G | T | T | T | C | T | poly | poly |
| Azerbaijan, 200m | 14163 | C | **R-A/G** | T | T | T | C | T | poly[a] | poly |
| Azerbaijan, 200m | 14164 | C | G | T | T | T | C | T | poly | poly |
| Azerbaijan, 200m | 14165 | **Y-C/T** | G | **Y-C/T** | C | **Y-C/T** | **Y-C/T** | **W-A/T** | **PxT(E)** | poly |
| Azerbaijan, 200m | 14166 | C | G | T | T | T | C | T | poly | poly |
| Azerbaijan, 200m | 14167 | C | G | T | T | T | C | T | poly | poly |
| Azerbaijan, 200m | 14168 | C | G | T | T | T | C | T | poly | poly |
| Azerbaijan, 200m | 14169 | C | G | T | T | T | C | T | poly | poly |
| Azerbaijan, 200m | 14170 | C | G | T | T | T | C | T | poly | poly |
| Azerbaijan, 200m | 14171 | C | G | T | **C** | T | C | T | **serav** | poly |
| Armenia, 1900m | 8761 | C | G | T | T | T | C | T | poly | poly |
| Armenia, 1900m | 8762 | C | **A** | T | T | T | C | T | poly | poly |
| Elburz Mtn., Iran, 2033m | 12603 | C | G | T | T | T | C | T | poly | poly |
| Elburz Mtn., Iran, 2033m | 12604 | C | G | T | **C** | T | C | T | **serav** | poly |
| Lushan, Iran, 1120m | 12789 | C | G | T | **C** | T | C | T | **serav** | poly |
| Hastjin, Iran,1610m | 12795 | C | G | T | **C** | T | C | T | **serav** | poly |
| Qushchi, Iran, 1760m | 12798 | C | G | T | **C** | T | C | T | **serav** | poly |
| Pakistan, seravschanica | 8483 | C | G | T | **C** | T | C | T | **serav** | serav |
| Pakistan, seravschanica | 8484 | C | G | T | **C** | T | C | T | **serav** | serav |
| Kazakhstan, seravschanica | 8224 | C | G | T | **C** | T | C | T | **serav** | serav |
| Kazakhstan, seravschanica | 8225 | C | G | T | **C** | T | C | T | **serav** | serav |
| Turkmenistan, turcomanica | 8757 | A | G | C | C | C | T | A | turc=exc | turco |
| Turkmenistan, turcomanica | 8758 | A | G | C | C | C | T | A | turc=exc | turco |

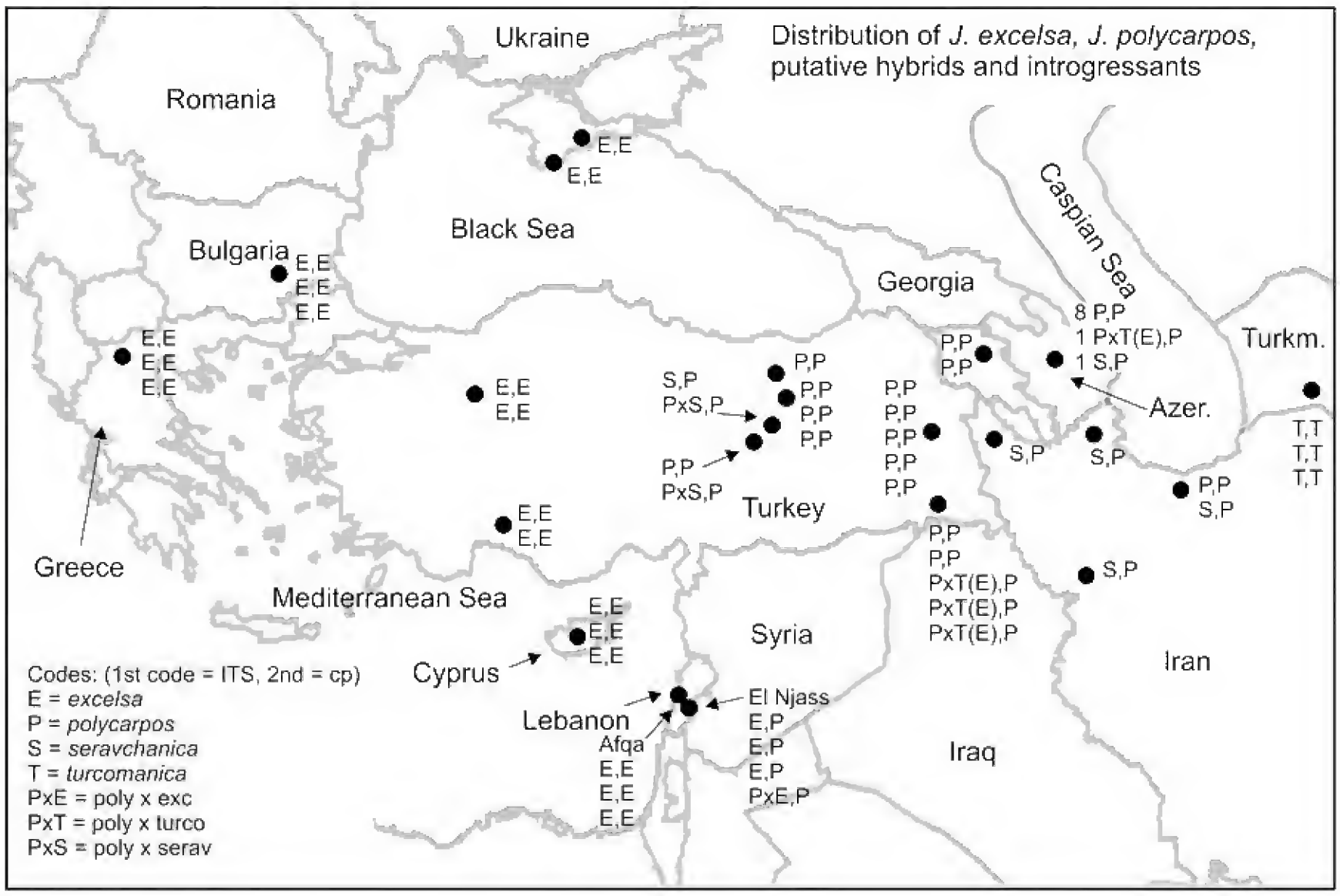


Figure 3. Distribution of *J. excelsa, J. polycarpos*, putative hybrids and introgressants based on ITS and cp sequences. The pair of capital letters (eg., E,E) gives the sample classification based on ITS (1st letter) and cp (2nd letter).

The situation in Lebanon seems to favor hybridization between *J. excelsa* and *J. polycarpos*, even though no pure *J. polycarpos* was found (Adams et al., 2014). Note the four plants in the El Njass population all have *J. polycarpos* cp, and one appears to be a ExP hybrid by nrDNA (Fig. 3). It seems likely that pure (P,P) *J. polycarpos* grows in the region, although these plants may have become extinct, in which case, this may be relictual hybridization.

Liao (1999), in a seminal paper on concerted evolution, defined it as "The molecular process that leads to homogenization of DNA sequences belonging to a given repetitive family". Liao reasoned that because rRNA functions only when assembled into large complexes, homogeneity of rRNAs is critical if all the steps of ribosome assembly and translation are to proceed normally. Liao (1999) says "One can therefore envision that a possible biological function of concerted evolution is to maintain homogeneous gene copies in a family so that homogeneous transcripts can be produced". However, it seems possible that there could be "silent" base substitutions that do not impact the shape or function of a rRNA. If so, these "silent" changes might persist indefinitely in a derived taxon.

Naciri and Linder (2015) estimated that typical tree species with $N_e$ of 1 million individuals and a generation time of 10 yrs would require 50 M yr after speciation to reach full monophyly. Syring et al. (2007) concluded that the presence of shared nrDNA haplotypes among *Pinus* species was due to incomplete lineage sorting. They estimated that reciprocal monophyly will be more likely than paraphyly in 1.7 to 2.4 M yr, but complete genome-wide coalescence in species could take up to 76 M yr.

However, Bouillé and Bousquet (2005) examined trans-specific allelic polymorphism in three low-copy nuclear genes in different *Picea* species and they estimated that allelic coalescence time between randomly selected alleles in *Picea* was 10 to 18 million years ago. The effective population size can greatly effect coalescence times (Naciri and Linder, 2015), such that species with smaller effective population sizes would coalescence faster than species with larger effective population sizes.

Mao et al. (2010) published an ancestral reconstruction of *Juniperus* based on all three (3) known *Juniperus* fossils. They showed *J. excelsa* and *J. polycarpos* joined in a clade at approximately 7 M yr. If that result is correct, then the amount of time available for complete nrDNA coalescence in the *J. excelsa* - *J. polycarpos* clade seems insufficient, compared with the 10 to 18 M yr required in *Picea* species (Bouillé and Bousquet, 2005). The ca. 7 M yr in *J. excelsa/ polycarpos* is far less than the 76 M yr that Syring et al. (2007) suggested was needed for coalescence of nrDNA in *Pinus*. It is difficult to know how accurate the dates are in Mao et al. (2010) due to the very small number (3) of *Juniperus* fossils used. But, it does appear that there has been insufficient time for complete coalescence of nrDNA in the present day *J. excelsa/ J. polycarpos*. Incomplete lineage sorting would explain the presence of *J. seravschanica* (S) nrDNA in otherwise, typical *J. polycarpos* in central-eastern Turkey, Azerbaijan and northwest Iran (Fig. 3).

The currently understood distributions of *J. excelsa, J. polycarpos, J. seravschanica* and *J. p.* var. *turcomanica* are depicted in Figure 4. The dashed line in central Turkey indicates the boundary between *J. excelsa* and *J. polycarpos* is unknown at present. The population of *Juniperus* on the north coast of the Black Sea may be *J. excelsa* or *J. polycarpos*. Recent events have made it impossible to collect in that area.

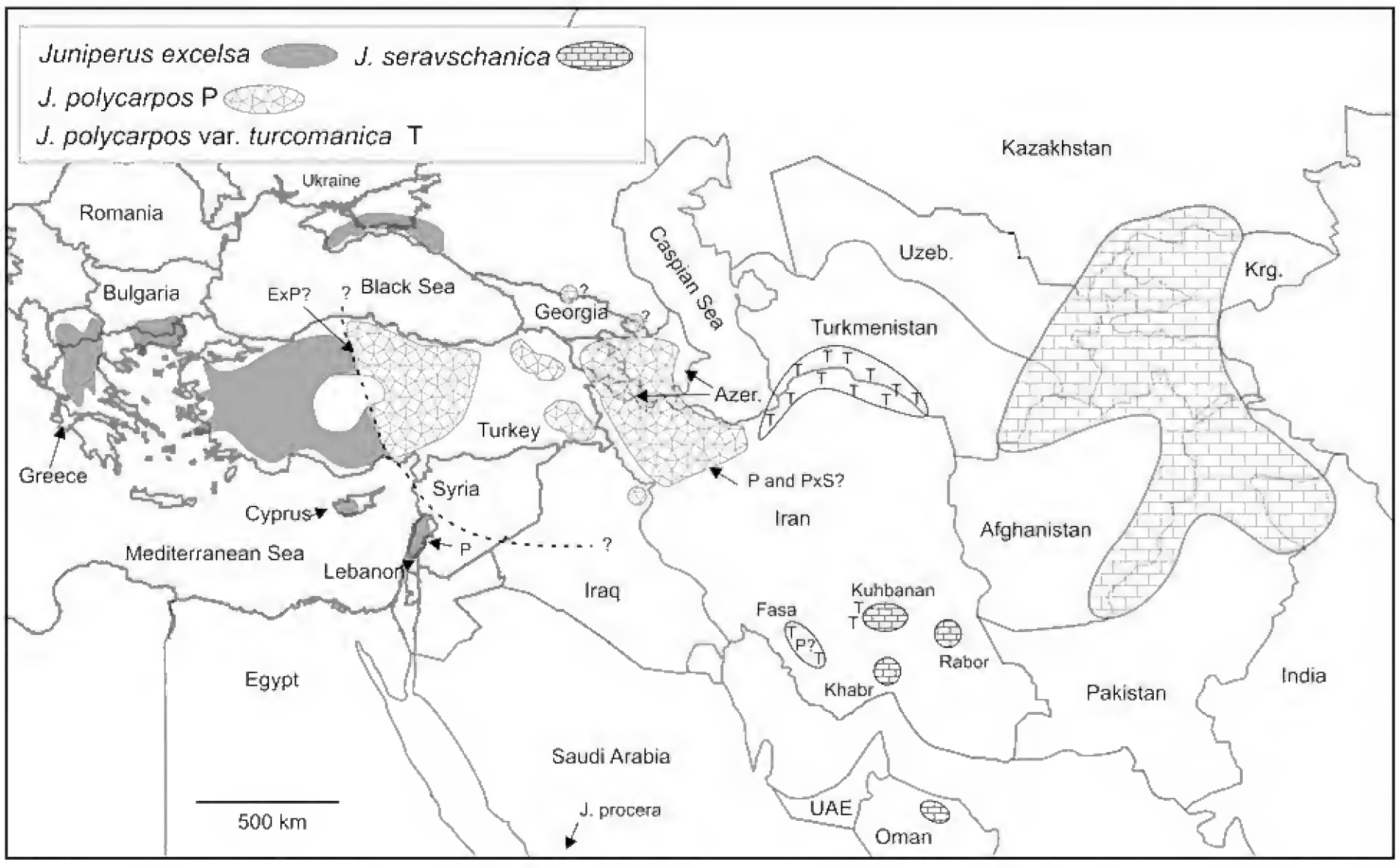


Figure 4. Distributions of *J. excelsa, J. polycarpos, J. p.* var. *turcomanica and J. seravschanica* as understood at present. The dashed line indicates the uncertain limits of *J. excelsa* and *J. polycarpos* in central Turkey. See text for discussion.

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## LITERATURE CITED

Adams, R. P. 1975. Statistical character weighting and similarity stability. Brittonia 27: 305-316.

Adams, R. P. 2013. Correction to Adams and Schwarzbach, Taxonomy of the multi-seeded, entire leaf taxa of the *Juniperus*, section *Sabina*: sequence analysis of nrDNA and four cp DNA regions. Phytologia 95: 226-227.

Adams, R. P. 2014. The junipers of the world: The genus *Juniperus*. 4th ed. Trafford Publ., Victoria, BC.

Adams, R. P., J. A. Bartel and R. A. Price. 2009. A new genus, *Hesperocyparis*, for the cypresses of the new world. Phytologia 91: 160-185.

Adams, R. P., B. Douaihy, M. D. Dagher-Kharrat, A. E. Schwarzbach and V. Farzaliyev. 2014. Geographic variation in nrDNA and four cpDNAs sequences of *Juniperus excelsa* and *J. polycarpos* in Greece, Turkey. Lebanon and Azerbaijan. Phytologia 96: 89-95.

Adams, R. P. and F. Hojjati. 2012. Taxonomy of *Juniperus* in Iran: Insight from DNA sequencing. Phytologia 94: 219-227.

Adams, R. P., F. Hojjati and A. E. Schwarzbach. 2014. Taxonomy of *Juniperus* in Iran: DNA sequences of nrDNA plus three cpDNAs reveal *Juniperus polycarpos* var. *turcomanica* and *J. seravschanica* in southern Iran. Phytologia 96: 19-25.

Adams, R. P. and M. E. Kauffmann. 2010. Geographic variation in nrDNA and cp DNA of *Juniperus californica, J. grandis, J. occidentalis* and *J. osteosperma* (Cupressaceae). Phytologia 92: 266-276.

Adams, R. P., J. A. Morris and A. E. Schwarzbach. 2008. Taxonomic study of *Juniperus excelsa* and *J. polycarpos* using SNPs from nrDNA and cp trnC-trnD plus essential oils and RAPD data. Phytologia 90: 208-225.

Adams, R. P. and A. E. Schwarzbach. 2012. Taxonomy of the multi-seeded, entire leaf taxa of *Juniperus* section Sabina: Sequence analysis of nrDNA and four cpDNA regions. Phytologia 94: 350-368.

Adams, R. P., S. Gucel, T. Mataraci, A. N. Tashev, B. Douaihy and M. D. Dagher-Kharrat, V. Farzaliyev and A. E. Schwarzbach. 2016. Geographic variation in nrDNA and four cpDNA regions of *Juniperus excelsa*: Analysis of new records from Bulgaria, Cyprus and southwestern Turkey. Phytologia 98: 1-7.

Bouillé, M., and J. Bousquet. 2005. Trans-species shared polymorhisms at orthologous nuclear genes loci among distant species in the conifer genus Picea (Pinaceae): Implications of the long-term main

Douaihy, B. G., G. Restoux, N. Machon and M. B. Dagher-Karrat. 2013. Ecological characterization of the *Juniperus excelsa* stands in Lebanon. Ecologia Mediterranea 39: 169-180.

Douaihy, B. G., G. Vendramin, A. Boratynski, N. Machon and M. B. Dagher-Karrat. 2011. High genetic diversity with moderate differentiation in *Juniperus excelsa* from Lebanon and the eastern Mediterrean region. AoB PLANTS 2011 pir003 doi:10.1093/aobpla/pir003.

Farjon, A. 2005. A monograph of Cupressaceae and *Sciadopitys*. Royal Botanic Garderns, Kew Press, London.

Farjon, A. 2010. A handbook of the world's conifers. Vol. I. Koninklijke Brill NV, Leiden, The Netherlands.

Liao, D-Q. 1999. Concerted evolution: Molecular mechanism and biological implications. Am. J. Hum. Genetics 64: 24-30.

Mao, K-S., G. Hao, J-Q. Liu, R. P. Adams and R. I. Milne. 2010. Diversification and biogeography of *Juniperus* (Cupressaceae): Variable diversification rates and multiple intercontinental dispersals. New Phytologist 188: 254-272.

Naciri Y. and H. P. Lindler. 2015. Species delimitation and relationships: The dance of the seven veils. Taxon 64: 3-16.

Posada, D. and K. A. Crandall. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14: 817-818.

Ronquist, F. and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572-1574.

Syring, J., K. Farrell, R. Businsky, R. Cronn and A. Liston. 2007. Widespread genealogical nonmonophyly in species of *Pinus subgenus Strobus*. Syst. Biol. 56: 163-181.

Veldman, D. J., 1967. Fortran programming for the behavioral sciences. Holt, Rinehart and Winston Publ., NY.